



(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 753 309 A2

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
15.01.1997 Bulletin 1997/03

(51) Int. Cl.⁶: **A61K 38/40**
// (A61K38/40, 31:16),
(A61K38/40, 31:47)

(21) Application number: **96830376.8**

(22) Date of filing: **03.07.1996**

(84) Designated Contracting States:
CH DE DK ES FR GB IT LI NL SE

(30) Priority: **12.07.1995 IT RM950472**

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(54) **Preparation of lactoferrin (or analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) for the therapy of viral infectious diseases**

(57) The present invention relates to the therapeutic utilization of the preparation of lactoferrin and desferrioxamine methanesulfonate for the therapy of many acute or recurrent viral infectious diseases in humans and animals. In details, the present invention demonstrates the antiviral activity, based on the inhibition either of the absorption either of the replication of several virus, possessed by a preparation of lactoferrin (or its analogous proteins like transferrins) in apo or iron or other metal ions saturated forms, together with desferrioxamine methanesulfonate (or other metal ion chelators like 8-hydroxyquinoline 1,10-phenanthroline, phosphonoacetic acid). This antiviral activity is well evident towards DNA virus; like *Herpesviruses*, and towards RNA virus, like *Rhinovirus*, and can be generally extended and utilized for the therapy of many acute or recurrent viral infections concerning skin, mucosae or other tissues.

EP 0 753 309 A2

Description

TECHNICAL FIELD

The present invention relates to the therapeutic utilization of a preparation of lactoferrin (or analogous proteins) in association with the iron chelator desferrioxamine methanesulfonate (or other metal ion chelators) for preventing and curing many viral infectious diseases in humans.

BACKGROUND ART

It is well known that virus can multiply at expenses of energy processes of the host cells. It is also well known that the first step of a viral infectious disease involves the binding of the viral particles to specific structures in the membrane of the host cell. These early interactions are followed by the intracellular phase of the virus consisting in the viral integration in the cell genome or the viral replication causing the lysis of the cell and the spreading of the infection. The integration of the viral genome in the cell genome allows the virus to avoid the host immune defenses and does not cause the lysis of the host cell as long as the viral genome remains integrated without multiplying. In some circumstances, the integrated viral DNA can start a new replication cycle, causing the cell lysis and, therefore, recurrent infections.

The peculiar life of virus makes difficult to find substances with antiviral properties able to prevent the replication of these pathogen agents without affecting the host cell. Furthermore, the antiviral drugs generally reduce the multiplication and the spreading of the virus in acute infections whereas are not able to avoid viral adsorption to host cells. For example, the infectious disease by *Rhinovirus* lack a suitable pharmacological therapy.

In order to inhibit the viral replication, an approach can be represented by hindering the early interactions virus-cell preventing the virus internalization or inhibiting the inactivating synthesis of the viral enzymes necessary for the multiplication cycle.

Concerning the first therapeutic approach, i.e. the inhibition of the early interaction virus-cell, many substances have been testing in the last years but just some of them are suitable to act through specific binding sites either to cell receptor either to viral anti-receptor. Recently, it has been demonstrated an antiviral function of lactoferrin, a glycoprotein able to bind 2 atoms of Fe^{3+} per molecule.

The antiviral function of lactoferrin, that was independent to this iron chelating property, is related to a specific binding with the host surface. In fact, it has been demonstrated that lactoferrin (human and bovine) specifically binds to heparan-sulphate, a common proteoglycan which is a multifunctional constituent of mammalian cell membranes and of extracellular matrix. Heparan-sulfate is also involved in the receptorial site that plays a role in the early interactions of HSV1 and other virus. Thus lactoferrin is able to selectively inhibit the early interactions between virus and host cell and, consequently is able to prevent the internalization.

In vivo and in the common media for cell cultures, lactoferrin is partially saturated in its iron binding sites. In this condition, lactoferrin assumes a "close" shape that allows it to better bind the surface receptors of the host cells. This binding between iron saturated lactoferrin and the receptor inhibits the adsorption of virus to the host cell while allows Fe^{3+} ions to penetrate inside the cell. Successively, the release of chelated ferric ions could prevent a therapeutic use of lactoferrin as antiviral agent, possibly because the iron transported into the cell (necessary for the viral replication) facilitates the multiplication of the virus particles eventually present in the cells.

The inhibition of the viral replication, is an example of the second therapeutic approach. It has been widely demonstrated that the activity of the viral replication is correlated to the presence of metal ions such as Fe^{3+} , Zn^{2+} , Cu^{2+} and several molecules, known to be metal ion chelators, possess antiviral activity. In particular, chelators like 8-hydroxyquinoline, 1,10-phenanthroline, phosphonoacetic acid and desferrioxamine methanesulfonate are able to inhibit the viral multiplication. From the above chelators, desferrioxamine metansulfonate, an hydroxamic siderophore produced by *Streptomyces pilosus*, utilized in the therapy of hemochromatosis, appears to be very active since it is able to chelate with high affinity intracellular Fe^{3+} ions having low affinity towards other metal ions.

Until now, the therapeutical antiviral utilization of these chelators was not made possible because these molecules, even if they are able to slow down the viral multiplication but do not prevent the penetration of the virus into the cell.

Herpesviridae is a family of viruses that are responsible of acute and recurrent infectious diseases. In particular, *Herpes simplex virus 1* (HSV1), is a virus that affects up to 90% of adults. The virus, after the first infection that is often clinically silent, can remain in latent form in skin and nervous cells. The virus is responsible of diseases such as lips or genital infection, primary gingival stomatitis, herpetic keratitis and umbilical skin infections. After events like exposure to sunlight, fever or stress, the virus can reactivate itself causing the appearing of painful and boring skin vesicles that hurt for several days even when the triggering cause is disappeared. Main locations of the recurrent infection are lips, genitals, face and sacrum.

At the moment, the treatments in use for the therapy of *Herpes simplex* infections are preventive (sunlight filters on lips) or antiviral (chemotherapeutic drugs inhibiting the viral replication). However, the first one prevents just the viral reactivation after sunlight exposure and the latter is potentially toxic and just reduces the intensity and length of symp-

toms but is not able to prevent the recurrence of the infections.

Rhinovirus are casual agents of the common cold. For this virus it is not possible the formulation of an effective vaccine, because of the 115 antigenic varieties found. The relative harmlessness of the above viral infections does not suggest the therapeutic utilization of antiviral drugs that always possess toxicity towards the host.

5

DISCLOSURE OF THE INVENTION

The present disclosure concerns the preparation of a mixture of at least one transferrin like lactoferrin (and, in a more general way, of proteins analogous to lactoferrin) and of at least one metal ion chelators such as desferrioxamine methanesulfonate or 8-hydroxyquinoline, 1,10-phenanthroline, phosphonoacetic acid, whose antiviral activity, that is here demonstrated to be exerted through the inhibition of adsorption and replication of several viruses, can be therapeutically utilized.

The therapeutic model, described in the present invention is based on the synergistic antiviral action of lactoferrin (or analogous proteins) and desferrioxamine metansulfonate (or other metal ion chelators). The antiviral activity of this association, not yet described, is much higher than that showed separately by the two components because their antiviral activity is exerted on two different phases of the viral cycle (adsorption and replication). It, therefore, makes obvious the therapeutic benefit of such an association towards viral infectious diseases in comparison with the common antiviral therapies that are focused on the inhibition of viral replication and show toxic effects towards the host organism.

Lactoferrin, which possesses very low or no toxic effect, and the chelator desferrioxamine methanesulfonate, already utilized in the human therapy, can be used at topic and systemic level against viral infections concerning skin and mucous membranes like nasal, oropharynx mucosa, intestinal, bronchial, vaginal and other mucosas.

As an example, we showed the antiviral activity towards DNA and RNA viruses, possessed by the preparation of lactoferrin (or its analogous proteins) and desferrioxamine metansulfonate (or other metal ion chelators). The antiviral activity is made clear by the protection of cell lysis due to viral replication of HSV1 (choosed as DNA virus) and *Rhinovirus* (choosed as RNA virus). The antiviral activity of the preparation of lactoferrin (or its analogous proteins) and desferrioxamine metansulfonate (or other metal ion chelators), is made evident by the absence or reduced presence of lysis plaques when the virus is added to cells cultured in the presence of this association. Since the antiviral activity of the preparation of between lactoferrin (or its analogous proteins) and desferrioxamine metansulfonate (or other metal ion chelators) is exerted towards either DNA viruses (like HSV1) either RNA viruses (like *Rhinovirus*.), is pointed out the possibility of an utilization of this association also for preventing and treating other acute or recurrent viral infections.

WAYS OF CARRYING OUT THE INVENTION

A particularly preferred preparation according to the invention is a mixture of the two compounds mentioned above, and thus a mixture of lactoferrin at a concentration of 15 to 99% methansulfonate and desferoxamine at a concentration at 1 to 95% these concentration being given by weith respect to the mixture. By way of example, a preparation according to the invention can comprise 98% lactoferrin and 2% desferoxamine methansulfonate.

Lactoferrin from bovine milk is the particularly preferred protein according to the invention. It appears clear, from the example reported below, that bovine lactoferrin can be considered optimum, with respect to treatments currently in use for the prevention and cure of viral infectious diseases although can be substituted by recombinant human lactoferrin in the case of systemic treatments. Furthermore, the extremely low toxicity of said protein contained in the formula according to the present invention is well-known, since is a "natural" substance extracted from bovine milk and is very similar, in its amino acid composition and structural configuration, to the homologous protein that is normally present in humans. This is an additional advantage of bovine lactoferrin with respect to the chemicals currently used in infectious treatments. The protein that is used according to the present invention can be extract or can be obtained by recombinant-DNA technology or also by chemical synthesis.

The term lactoferrin designates a glycoprotein present in colostrum and milk and in many biological secretions and in the leucocyte granules of mammals. Lactoferrin possesses an isoelectric point of 7.8, a molecular weight of about 83,000 Da and two sugar chains. Each molecule of lactoferrin binds 2 atoms of Fe^{3+} in presence of sodium bicarbonate. The main function of this protein is to reduce the amount of free iron in biological liquids, inhibiting the bacterial growth (the bacteriostatic property of lactoferrin is already well known) and decreasing the risk of forming free radicals due to the presence of not chelated iron. Lactoferrin belongs to a family of glycoprotein generally called "Transferrin", characterized in so that they possess two binding sites for Fe^{3+} per molecule and a high degree of sequence homology among them.

Lactoferrin can be industrially produced by purification from bovine milk. We mainly used bovine lactoferrin in the experiments described in the present disclosure but the same antiviral activity is possessed by all the marketable proteins known as lactoferrin such as human lactoferrin and lactoferrin from mouse, produced by extraction or by recombinant DNA techniqu , and, therefore, the antiviral activity of lactoferrin has to be extended, as described in the present disclosure, to all the lactoferrins from various natural or synthetic sources.

Furthermore, we tested different Fe^{3+} , Cu^{2+} , Mn, Ni, Zn saturation percentage of the above glycoproteins, from Apo form to totally metal saturated form. All lactoferrin preparations possessing different metal saturation have showed similar antiviral activity when these preparations are tested alone or in association with desferrioxamine methanesulfonate or other metal ion chelators.

5 The antiviral activity towards viruses possessed by the preparation of lactoferrin and desferrioxamine methanesulfonate (or other metal ion chelators), described in this disclosure, is also possessed, even with a lower antiviral activity, by other proteins chemically and enzymatically modified and belonging to the "Transferrin family" as lactoferrin substitutes.

10 The term desferrioxamine methanesulfonate designates a metabolite produced by *Streptomyces pilosus* able to chelate 1 Fe^{3+} per molecule also in the intracellular space. Under a chemical point of view, desferrioxamine methanesulfonate belongs to the trihydroxamic siderophore class that can bind with high affinity Fe^{3+} ions and with lower affinity other metal ions like Al^{3+} , Cu^{2+} , Zn^{2+} and Co^{2+} .

15 The antiviral activity of the preparation of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) described in the present disclosure is showed at not toxic concentrations of lactoferrin and desferrioxamine methanesulfonate towards cultured cells of different sources.

The antiviral activity towards DNA and RNA virus possessed by the association of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) is the main claim of this disclosure while it is not relevant, as regard with the antiviral activity towards virus described in this disclosure, the source of the lactoferrin and desferrioxamine methanesulfonate.

20 The formula according to the present invention can be obtained and stored in liquid form, as solutions at a concentration of 0.1 to 10%, e.g. 1 to 5%, weight/volume (g/ml) of the mixture in solvents acceptable for pharmaceutical use, in particular water or hydroalcoholic solvents such as water-ethanol mixtures, or in solid form (lyophilized, dried, frozen) and in the other commonly known forms of storage: for example immobilized or adsorbed on an inert support commonly used in the pharmaceutical field.

25 The formula according to the invention, can thus be used in the liquid form, or in solid form, with the concentration specified above.

In its form ready-for use the preparation according to the invention, can also comprise further conventional compounds as well as carriers, fillers, flavouring agents, preservatives, surfactants, colorants and other adjuvants selected from those conventionally used for the various liquid or solid form preparations.

30 The formulation, according to the invention, can comprise antibacterial compounds such as quaternary ammonium compounds with one long chain alkyl on the nitrogen atom, alkali metal pyrophosphates and orthophosphates, halogenated bisphenols and halogenated diphenyl ethers, sodium benzoate, sodium salicylate, etc.

35 The formulation, according to the invention, can further comprise diluents, e.g. glycerin, propylene glycol, dioxalanes, glycerol, glycofurol, dimethylacetamide, ethyl lactate, alcohols, glycols, sorbitols, USP oils, NF oils, lecithin, lanolin, ethyl oleate, isopropyl myristate, benzyl benzoate, petrolatum, cocoa butter mixts and gelifying substances e.g.: carboxy vinyl polymers, sodium alginate, agar, gelatin, cellulose derivatives, pectin, xantan gum, polyvinyl pyrrolidone, glycerol, propylene glycol or polyethylene glycol.

40 The formulation, according to the invention, in either ointment, gel powder, solution should be used for purposes of prevention of recurrent viral diseases, preferably twice a day. For purpose of treatment the frequency of use can be increased to 3 to 4 times a day.

The preparation of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) has been tested for its antiviral activity towards HSV1, as prototype pathogen DNA virus, through the inhibition of very early phases of the adsorption and viral replication following an experimental model described below.

45 The preparation of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) has been also tested for its antiviral activity towards *Rhinovirus* as prototype pathogen RNA virus, using an experimental model described below.

50 The utilization of the association of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) in the therapy of infections by HSV1 and *Rhinovirus* are, therefore, two of the possible therapeutic applications of the antiviral activity of the preparation of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators), described in this disclosure.

In fact, the antiviral properties of the preparation of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) can make this association also valid for the topic or systemic therapy of many acute and recurrent infectious diseases by DNA and RNA pathogen virus concerning humans and animals.

55 The here reported examples point out that the therapeutic antiviral activity towards different viruses possessed by the preparation of lactoferrin (or its analogous proteins) and desferrioxamine methanesulfonate (or other metal ion chelators) described in the present disclosure, can be utilized in acute or recurrent viral infections and can be considered optimal as regards the commonly used therapeutic treatments because its very low or no toxic effects.

The following examples have been carried out on *in vitro* models that are considered valid for testing substances with antiviral activity.

EXAMPLES

Cell cultures

The cultured cell utilized are different according with the tested virus. For testing HSV1, VERO cells have been utilized while for testing *Rhinovirus* we utilized HeLa cells. Both cell lines have been cultured as monolayers at 37°C in Eagle's MEM containing 1.2 g/l NaCO₃, 2 mM glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 10% heat inactivated faetal calf serum in 5% CO₂ on 24- well tissue culture clusters (costar) by loading 0.5 ml of a suspension of 2x10⁵ HeLa cells/ml.

Unless otherwise indicated, lactoferrin and ovotransferrin used in the following examples are 30 % iron-saturated.

Example 1

Activity of lactoferrin and desferrioxamine methanesulfonate and related substances on the very early Interactions between cell and viruses belonging to different genera.

The utilized procedure is the following:

- a) incubation at 4°C for 1 h of the tested substances with cultured cells. After this period, the monolayer is washed and incubated at 37°C to allow viral infection;
- b) incubation at 4°C for 1 h of the tested substances with the tested viruses. After this period, the monolayer is washed and incubated at 37°C to allow viral infection;
- c) incubation at 4°C for 1 h of the tested substances with the virus and the cell monolayers. After this period, the monolayer is incubated at 37°C to allow viral infection.

The data concerning the activity of the lactoferrin (or other substances) are expressed as percentage of lysis plaques in comparison with the control in which the virus is incubated in its (or their) absence. The cultured cell utilized are different according with the tested virus. For testing HSV1, VERO cells have been utilized, while for testing *Rhinovirus* we utilized HeLa cells. Both cell lines have been cultured as monolayers at 37°C in Eagle's MEM containing 1.2 g/l NaCO₃, 2 mM glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 10% heat inactivated faetal calf serum in 5% CO₂ on 24- well tissue culture clusters (costar) by loading 0.5 ml of a suspension of 2x10⁵ HeLa cells/ml.

Lactoferrin and glycosilate ovotransferrin have been used at the concentration of 1 mg/ml; desferrioxamine and 8-idrossiquinoline at the concentrations of 0.03 mg/ml.

Table 1

Effect of transferrins and chelators on the very early cell-virus interactions.			
Conditions		Plaque forming units (%)	
		HSV1	RHINOVIRUS
Control		100	100
Bovine lactoferrin	a	10	20
	b	0	10
	c	0	5
Ovotransferrin	a	100	100
	b	100	100
	c	90	80
Glycosilate ovotransferrin	a	30	20
	b	10	10
	c	10	10
Desferrioxamine methanesulfonate	a	50	80
	b	100	100
	c	40	60
8-hydroxyquinoline	a	60	70
	b	100	100
	c	50	70

In Table 1 are reported the data relative to bovine lactoferrin, in this experimental conditions, more active than human lactoferrin. On the contrary, ovotransferrin shows a poor antiviral activity except when it is chemically increased its glycosilation degree. Concerning the inhibition of the very early cell-virus interactions, desfemoxamine methanesulfonate, like 8-hydroxyquinoline and other described chelators, a does not possess the same activity.

Example 2

Activity of transferrins and chelators on the replication of viruses belonging to different genera.

These experiments have been performed adding the tested substances at 37 °C to the monolayer preincubated for 1 h at 4°C in the presence of the different viruses. The data are reported in table 2.

Table 2

Effect of transferrins and chelators on the replication of different virus.		
Conditions	Plaque forming units (%)	
	HSV1	RHINOVIRUS
Control	100	100
Bovine lactoferrin	100	100
Ovotransferrin	100	100
Glycosilate ovotransferrin	100	100
Desferrioxamine methanesulfonate	80	90
8-Hydroxyquinoline	75	80

From the reported data, is evident that lactoferrin and other transferrins do not affect the intracellular phase and the replication of the viruses.

A light effect is observed just for desferrioxamine methanesulfonate and 8-hydroxyquinoline for their well known mechanism of binding the intracellular iron necessary for the viral activity.

Other experiments have been carried out using the same concentration of bovine lactoferrin and ovotransferrin completely saturated with different metal ions. The data are reported in Table 3.

Table 3

Effect of lactoferrin saturated with different metal ions on the replication of different virus.		
Conditions	Plaque forming units (%)	
	HSV1	RHINOVIRUS
Control	100	100
Bovine lactoferrin in apo form	30	30
Bovine lactoferrin iron saturated	0	0
Bovine lactoferrin zinc saturated	40	40
Bovine lactoferrin copper saturated	10	20
Bovine lactoferrin manganese saturated	0	0
Bovine lactoferrin cobalt saturated	50	30
Bovine lactoferrin nickel saturated	30	40

From the data reported in Table 3 it can be notice that lactoferrin also in saturated forms shows a noticeable antiviral activity.

Example 3

Activity of the transferrins-chelators association.

The maximum of the antiviral activity can be obtained when the substances (lactoferrin or glycosilate ovotransferrin and desferrioxamine methanesulfonate or 8-hydroxyquinoline) exert all together their action i.e. the inhibition of the virus-cell interaction by lactoferrin or glycosilate ovotransferrin and the inhibition of replication by desferrioxamine methanesulfonate or 8-hydroxyquinoline.

The experimental procedure is described below:

- a) incubation at 4°C for 1 h of different associations with cultured cells. After this period, the monolayer is washed and incubated at 37°C to allow viral infection;
- b) incubation at 4°C for 1 h of different associations with the tested viruses. After this period, the monolayer is washed and incubated at 37°C to allow viral infection;
- c) incubation at 4°C for 1 h of the different associations with the virus and the cell monolayers. After this period, the monolayer is incubated without washing at 37°C to allow viral infection.

Table 4

Activity of the transferrins-chelators associations.			
Conditions		Plaque forming units (%)	
		HSV1	RHINOVIRUS
Control		100	100
Bovine lactoferrin +desferrioxamine methanesulfonate	a	0	0
	b	0	0
	c	0	0
Glycosilate ovotransferrin +desferrioxamine methanesulfonate	a	0	0
	b	0	0
	c	0	0
Lactoferrin + 8-idroxiquinoline	a	0	0
	b	0	0
	c	0	0
Glycosilate ovotransferrin + 8-hydroxyquinoline	a	0	0
	b	0	0
	c	0	0

It is well evident that in all the experimental conditions the viral replication is totally inhibited. In fact, the transferrins-chelators association of is active either on the very early cell-virus interaction either on the intracellular replication of the viruses, so exerting the maximum of the antiviral effect. Similar result were also obtained with lactoferrin or ovotransferrin saturated with different metal ions such as Mn^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} .

Example 4 - Lyophilized powder

Composition per 5 g

Active ingredients:

Lactoferrin, from bovine milk, SIGMA Chemical Co., cat L4765, 4,8 g
Desferrioxamine methanesulfonate, Ciba Geigy, 0.2 g

Use

Dissolve into distilled water and inject i.m. once a day.

Example 5 - Ointment

Composition per 100 g

5 **Active ingredients:**

Lactoferrin, from bovine milk, SIGMA Chemical Co., cat L4765, 10 g
Desferroxamine methanesulfonate, Ciba Geigy, 0.1 g

10 **Carriers, preservatives agents:**

Paraffin oil 10 g
Vaseline 80 g

15 **Use**

Apply on the skin twice a day

Example 6 - Cream

20

Composition per 100 g

Active ingredients:

25 Lactoferrin, from bovine milk, SIGMA Chemical Co., cat L4765, 10 g
Desferroxamine methanesulfonate, Ciba Geigy, 0.1 g

Carriers, preservatives agents:

30 Paraffin oil 6 g
Chlorocresol 0.1 g
Vaseline 15 g
Ketostearil alcohol 7.2 g
Polyethylene glycol monocethyl ether 1,8 g
35 Distilled water up to 100 g

Use

Apply on the skin twice a day.

40

Example 7 - Gel

Composition per 100 g

45 **Active ingredients:**

Lactoferrin, from bovine milk, SIGMA Chemical Co., cat L4765, 10 g
Desferroxamine methanesulfonate, Ciba Geigy, 0.1 g

50 **Carriers, preservatives agents:**

55

EP 0 753 309 A2

Methyl-p-hydroxybenzoate	100 mg
Propyl-p-hydroxybenzoate	20 mg
Xantan gum	4 g
Lecithin	3 g
Vitamin E	100 mg
Distilled water up to	100 g

Use

Apply on the skin twice a day.

Example 8 - Spreading powder

Composition per 100 g

Active ingredients:

Lactoferrin, from bovine milk, SIGMA Chemical Co., cat L4765, 10 g

Desferroxamine methanesulfonate, Ciba Geigy, 0.1 g

Carriers, preservatives agents:

Glycine	4 g
Zin oxide	3.4 g
Corn starch	80 g
Sodium metabisulfite	100 mg
Silica	1 g

Use

Spread on the skin twice a day

Example 9 - Nasal spray

Composition per 100 g

Active Ingredients:

Lactoferrin, from bovine milk, SIGMA Chemical Co., cat L4765, 10 g

Desferroxamine methanesulfonate, Ciba Geigy, 0.1 g

Carriers, preservatives agents:

5

10

Methyl-p-hydroxybenzoate	100 mg
Propyl-p-hydroxybenzoate	20 mg
Sodium phosphate monobasic	200 mg
Sodium EDTA	50 mg
Distilled water up to	100 g

15

Use

Spray into the nostrils twice a day

20

Example 10 - Nasal drops

Composition per 100 ml

Active ingredients:

Lactoferrin, from bovine milk, SIGMA Chemical Co., cat L4765, 10 g
Desferroxamine methanesulfonate, Ciba Geigy, 0.1 g

Carriers, preservatives agents:

35

40

Methyl-p-hydroxybenzoate	100 mg
Propyl-p-hydroxybenzoate	20 mg
Sodium chloride	0.8 g
Distilled water up to	100 ml

Use

45

Drop 0.5 mls of solution per each nostril twice a day

Claims

- 50 1. Preparation for preventing and curing acute and recurrent viral infectious diseases concerning skin, mucosas and other human or animal tissues characterized in that it comprising at least one transferrin and at least one molecule selected from the groups of chelators of iron and other metal ions.
- 55 2. Preparation according to claim 1, wherein the transferrin, in apo or iron or other metal ions saturated forms, at a protein concentration of 5 to 99% is represented by the bovine or human lactoferrin or by other natural or chemically modified transferrin characterized by high sequence homology with the protein known as lactoferrin, for example glycosilate ovotransferrin.
3. Preparation according to claim 1, wherein the chelator of iron and other metal ions at a concentration of 1 to 95%

is represented by desferrioxamine methanesulfonate or by other molecules with similar metal ion binding capability, for example 8-hydroxyquinoline, 1,10-phenanthroline and phosphonoacetic acid.

4. Preparation according to claims 1 and 2, wherein said transferrin proteins are selected from the group consisting of said proteins of natural origin, comprising proteins of human, animal and plant origin, obtained by recombinant DNA technology and chemically synthesized.
5. Preparation according to claims 1 and 3 wherein said iron chelating molecules are selected from the group consisting of said molecules of natural origin and chemically synthesized molecules.
6. Preparation formed by the association of lactoferrin or analogous protein and desferrioxamine methanesulfonate or other metal ion chelator according to claims 1,2 and 3, 4 and 5 wherein it is utilized for preventing and curing acute and recurrent viral infectious diseases concerning skin, mucosae and other tissues of humans and animals caused by DNA virus for examples, those belonging to the *Herpesvirus* family.
7. Preparation formed by the preparation of lactoferrin, or analogous protein, and desferrioxamine methanesulfonate, or other metal ion chelator, according to claim 1,2 3, 4 and 5, wherein it is utilized for preventing and curing acute and recurrent viral infectious diseases concerning skin, mucosae and other tissues of humans and animals caused by RNA viruses for examples, those belonging to the *Picornavirus* family like *Rhinovirus*.
8. Preparation formed by the preparation of lactoferrin or analogous protein, and desferrioxamine methanesulfonate or other metal ion chelator according to claims 1,2,3, 4,5,6 and 7 wherein it is utilized in liquid, solid or immobilized form for preventing and curing viral infectious diseases.
9. Preparation according to claims 1,2,3,4,5,6 and 7 wherein said mixture is in a form further comprising one or more adjuvants selected from carriers, preservatives, diluents and gelling agents.
10. Methods of using a preparation according to claim 1 for preventing or treating viral infectious diseases of humans and animals, comprising topical or systemic administering said preparation at least once a day.